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Rare specimen identification in an un-integrated taxonomy: implications of DNA sequences from a Taiwanese *Philine* (Mollusca, Philinidae)

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Abstract

Many species of the gastropod genus *Philine* have been named from northeastern Asia but scanty descriptions based predominantly on shells make it difficult to determine which are valid. This, plus the sporadic anatomical and genetic information available for many of these species has led to what may be described as an un-integrated taxonomy. In this situation, it is generally preferable to postpone dissection of rare and unusual specimens until relevant diagnostic characters can be established in broader studies. Micro-CT scanning and DNA sequencing were used to examine such a specimen collected recently from deep waters off northeastern Taiwan. Micro-CT examination of the morphology of the internal shell and gizzard plates suggested that, among named species, the sequenced specimen is most similar to *P. otukai*. It cannot, however, be definitively referred to *P. otukai* as that species lacks adequate anatomical description or known DNA sequences. Phylogenetic analyses of newly collected DNA sequences show the specimen to be most closely related to, but distinct from the northern Atlantic Ocean and Mediterranean species, *Philine quadripartita*. The sequences also confirm genetically that five or more species of *Philine* occur in northeast Asia, including at least three subject to considerable taxonomic uncertainty.

Keywords

Gizzard plates, 16S ribosomal RNA, micro-CT scanning, Scaphopoda

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Introduction

Accurate delimitation of species of the highly speciose cephalaspidean genus *Philine* Ascanius, 1772 is hampered by the brief original descriptions of many of its named taxa. Many such descriptions were made using only the shell, which has limited diagnostic value, and often these have not been subsequently supplemented by studies of internal anatomy, including morphology of the diagnostically important gizzard plates, or DNA sequences.

The difficulty of delimitation is especially marked in northeastern Asia, as exemplified by the situation in Taiwan from which three named *Philine* species (*P. argentata* Gould, 1859; *P. vitrea* Gould, 1859, and *P. otukai* Habe, 1946) are currently recognised (Lee 1998; Wu 2004; Wu and Lee 2005). We have recently collected a specimen from deep waters off Taiwan that is not readily identifiable owing to the lack of an adequate taxonomic description of comparable species. A fourth species, *Philine kurodai* Habe, 1946, has been recorded in Taiwan but the relevant specimens were supposed to belong to *P. otukai* according to Lee (1998) and Wu (2004). Many other species of *Philine* have been named from nearby regions, and in total, 15 named recent species with medium to large shells have been recorded (Table 1) from the coasts of China, Korea, and Japan (Zhang 2008; Price et al. 2011; Chaban 2014; Chaban and Chernyshev 2014; Chaban et al. 2019; and references in these articles). The anatomical and genetic information available for most of these species is sporadic, resulting in a taxonomy that may be described as un-integrated, with a general lack of diagnostic characters suitable for species discrimination. In this situation, we consider it advisable to minimise destructive sampling of rare specimens until such diagnostic characters can be established from more frequently collected taxa.

We used non-destructive morphological analysis of the collected specimen and DNA sequencing of a small external tissue sample to make initial comparisons with named species. For morphological analysis, we used micro-CT scanning (Shepelenko et al. 2015) which is potentially very useful for studying specimens where dissection would be premature. We focussed on the shape and sculpture of the shell, which is internal in *Philine*, and the three gizzard plates which have been applied to morphological diagnoses of the genus (e.g., Price et al. 2011).

We determined sequences of 16S ribosomal RNA (16S rRNA), histone H3 (H3), and the D1 expansion region of 28S ribosomal RNA (D1 28S rRNA). We then performed phylogenetic analyses of the sequences, including sequences from previous DNA studies of the Philinidae (Krug et al. 2012; Ohnheiser and Malaquias 2013; Gonzales and Gosliner 2014; Oskars et al. 2015; Chaban et al. 2019). We were unable to sequence the cytochrome *c* oxidase subunit I bar-coding region, a difficulty also found by Krug et al. (2012), possibly explaining why there are relatively few GenBank accessions for this gene from *Philine*.

Table 1. *Philine* species from northeastern Asia with medium- or large-sized shells. Localities of identified type specimens are followed by (T). Information categories are abbreviated as: D, DNA sequence; G, gizzard plate morphology, O, other anatomical.

Species	Locality	Information	Status
P. acutangula Adams, 1862	Gulf of Lian-Tung, Hulu-Shan Bay, China [Gulf of Liadong, Hulushan Bay]	No figure or type description	Species inquirendum
P. argentata Gould, 1859	Hokkaido Bay, Japan (T)	G, O: Chaban (2014) D: Chaban et al. (2019)	Synonym of *P. orientalis* (Price et al. 2011); species status maintained by Chaban (2014)
P. coreanica Adams, 1855	Coreen archipelago on mud flats [Korea]	Sowerby (1855) fig. 166 (figured type?)	Species inquirendum: "*P. aperta*" fide Herdman (1906)
P. crenata Adams, 1862	Tsu-Shima, Korea Strait, 46 m		Species inquirendum: "*P. aperta*" fide Herdman (1906)
P. habei Valdés, 2008	Fiji, 17°05'S, 178°55'W, 654–656 m (T)	D: Oskars et al. (2015) G, O: Valdés (2008)	Accepted
P. japonica Lischke, 1872	Jedo (Tokyo Bay), Japan (T)	D: Chaban et al. (2019) O: Larvae: Hamatani (1961) and Tanaka (1958).	*P. orientalis* synonym (Price et al. 2011). Species status maintained by Chaban et al. (2019)
P. kinglipini Tchang, 1934	Bays of Tsangkou and Nukukou	G, O: Tchang (1934)	Accepted. No type designated.
P. kurodai Habe, 1946	Wakayama Prefecture, Japan (T)		Accepted by Chaban (2016)
P. miyadii Habe, 1946			Synonym of *P. scalpta* fide Habe (1950) (= *P. vitrea* (see below)
P. orientalis Adams, 1854	"Eastern seas" (T)	G, O: Price et al. (2011) D: Krug et al. (2012)	Accepted
P. otukai Habe, 1946	Penghu Islands, Taiwan (T)		Accepted
P. paucipapillata Price, Gosliner & Valdés, 2011	Kampote and Prek Romeas, Cambodia (T)	G, O: Price et al. (2011) D: Krug et al. (2012)	
P. scalpta A. Adams, 1862	Tsu-Shima and Korea Strait 50–80 m	D: Chaban et al. (2019)	Synonym of *P vitrea* (Higo et al. 1999). Species status maintained by Chaban and Chernyshev (2014).
P. striatella Tapparone-Canefri,1874	Yokohama, Japan (T)	O (radula): Tapparone-Canefri (1874)	Synonym of *P. japonica* fide Pilsbry (1895), or *P.orientalis* fide Price et al. (2011)
P. vitrea Gould, 1859	Hong Kong (T)		Accepted

Materials and methods

Material

AMS C.559479, off Dasi, Yilan County, north-east Taiwan, 24°54'N, 122°E, 5 xi 2015, coll. S. Ahyong, commercial bottom trawl, 300–400 m depth, fixed and stored in 80% ethanol. The specimen was collected together with other deep-water species including the crustaceans, *Bathynomus doederleini* Ortmann, 1894, *Homola orientalis* Henderson, 1888, and *Metanephrops thomsoni* (Bate, 1888).

Measurements

Digital callipers were used for external measurements, made after preservation. Measurements of the relative sizes of internal structures, such as gizzard plates, were deter-

mined from images using the beta 4.0.3 version of Scion Image (Scion Corporation, Frederick, MD, USA). Sizes of internal structures were estimated by multiplying the ratio of the relative lengths of the relevant structure and the longest shell axis on an image by the length of this axis determined by callipers on the actual specimen.

Micro-CT imaging

Micro-CT scanning was performed directly on the specimen after removal from ethanol storage with a Siemens Inveon micro-CT scanner operated at 80 KV energy, 250 μA intensity with 540 projections per 360° and 2200 ms exposure time. The sample was scanned at a nominal isotropic resolution of 27.8 μm. The data were reconstructed using a Feldkamp conebeam back-projection algorithm provided by an Inveon Acquisition Workstation from Siemens.

DNA methods and molecular phylogenetic analysis

DNA was extracted from a small amount of mantle tissue using the Bioline Isolate II Kit following the manufacturer's instructions. The final centrifugation was performed with 100 μL of the kit's elution buffer. PCR amplifications followed the methods of Colgan and Da Costa (2009) using the following primers:

16S rRNA:	16Sar CGCCTGTTTATCAAAAACAT (Palumbi 1996), 16sbr CCGGTCTGAACTCAGATCACGT (Palumbi 1996)
28S rRNA:	28S D1F ACCCSCTGAAYTTAAGCAT (McArthur and Koop 1999)
	28S D1R AACTCTCTCMTTCARAGTTC (Colgan et al. 2003)
H3:	H3NF ATGGCTCGTACCAAGCAGAC (Colgan et al. 2000)
H3NR	ATRTCCTTGGGCATGATTGTTAC (Colgan et al. 2000)

PCR products were checked by electrophoresis on 2% agarose gels run with TAE buffer, including Gel Red (Biotium Inc., Fremont, CA) and visualised with UV-fluorescence. Single-banded products of the appropriate size were purified by Exo-SAP-IT™ (Thermo-Fisher Scientific) and sequenced commercially at Macrogen (Seoul, Korea) with the primers used in the original amplification.

GenBank Accession Numbers for new sequences: MH340050 (D1 28S rRNA), MH340051 (16S rRNA), MH340052 (histone H3).

Phylogenetic analyses were conducted using all *Philine* sequences for each gene available on 20 September 2020. Sequences from *Philinopsis* Pease, 1860 were used to root analyses for the 16S rRNA dataset. The sets of sequences for each gene were aligned with ClustalW (Thompson et al. 1997).

Maximum likelihood analyses (ML) analyses were conducted at the CIPRES Science Gateway (https://www.phylo.org/portal2/home.action, Miller et al. 2010) using the RAxML Blackbox (Stamatakis et al. 2008), assuming no invariable sites, using empirical base frequencies and assessing node support by rapid bootstrap

with the number of replicates determined by the software, according to the MRE-based bootstopping criterion (Pattengale et al. 2010). Analyses of the complete 16S rRNA data were also run on a filtered alignment for which GBlocks (Castresana 2000) was used to remove less well supported sections, allowing gap positions within the final blocks and less strict flanking positions. Analyses were also conducted for a reduced set of 16S rRNA sequences comprising members of a strongly supported clade of taxa allied with *P. aperta*. MEGA 7.0 (Kumar et al. 2016) was used to conduct other analyses including calculation of genetic distances and minimum evolution phylogenetic trees.

Results

Morphology

Animal, external: (Fig. 1) **Colour.** headshield creamy yellow, parapodia paler, foot off-white. Headshield: anterior three-fifths transversely rugose; central anterior to posterior furrow more marked posteriorly; dividing into two short processes posteriorly, overlapping posterior shield very slightly; tapering posteriorly in dorsal view (length 15.6 mm, width at widest 12.2 mm, width at shell 9.00 mm). Parapodial lobes broad, not remarkably thickened. Foot extending slightly posterior to shell. Posterior shield mostly occupied by a thinly covered internal shell. Posterior shield appearing grey-black due to pigmentation of internal organs, maintained after fixation. **Dimensions:** Animal 35.3 mm long (buccal mass everted) and 21.5 mm wide when newly collected (measured from photograph). After fixation: length from front of headshield to tail of foot 34 mm, width 19.8 mm and maximum height 9.3 mm.

Shell: (Fig. 2) fragile, internal, height of body whorl, measured vertically from the posterior end of the aperture to the tip of the (damaged) elevated anterior outer lip: 18.4 mm, width of body whorl 14.2 mm, measured by callipers; spire involute, aperture extremely wide and rounded below, upper margin rising above shell vertex, columellar lip notably reflected, albeit slightly, (Fig. 2A). Frequent irregularly-spaced curved growth lines and spiral ribs, covering most of body whorl (except for bottom quarter). Radial and spiral ribs sometimes intersecting to form weak, irregularly cancellate sculpture (Suppl. material 2: Fig. S1).

Gizzard plates: (Figs 2B–D, 3). One pair of similar plates (12.5 mm in longest dimension) and notably smaller, unpaired plate (9.0 mm in longest dimension), all with outer surface (that facing towards the animal's surface) slightly dimpled. Plates spindle-shaped, each with two large, circular pores in central area markedly depressed inwards. Smallest plate with spindle arms aligned axially; axes of spindle arms of larger plates form an obtuse angle (~ 135°). Spindle arms unequal in length (measured from the midpoint of the line between the two pores in each plate). Ratio of arm lengths: 1.15 for the small plate; 1.18 for paired plates. Posterior arm of all plates more slender than anterior arm.

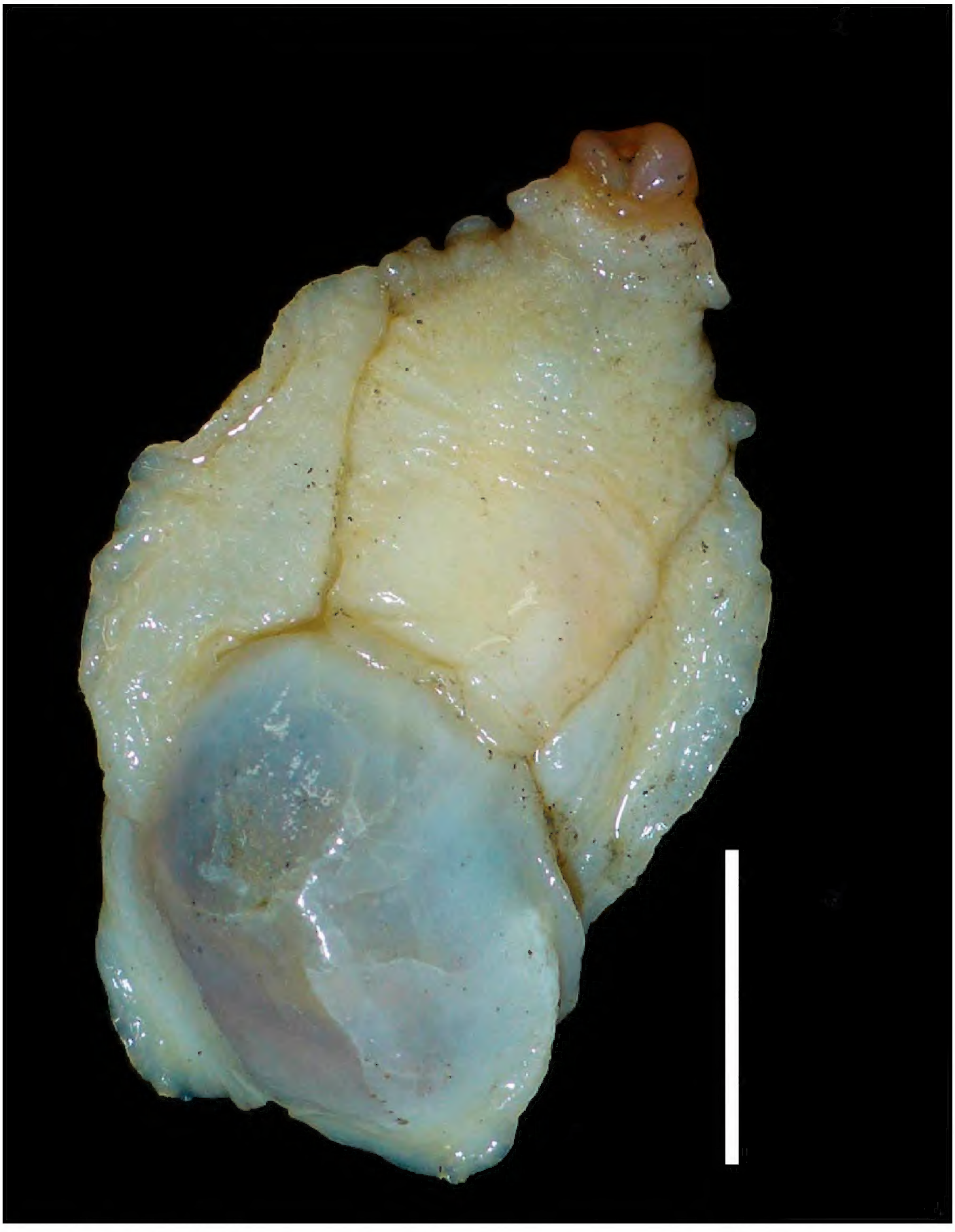

Figure 1. C.559479, freshly caught animal (photograph Shane Ahyong). Scale bar: 10 mm.

Gut contents

The prey of *Philine* are predominantly bivalves (Morton and Chiu 1990) but gastropods are also taken. For example, *P. orientalis* is known to prey on at least 11 species of small

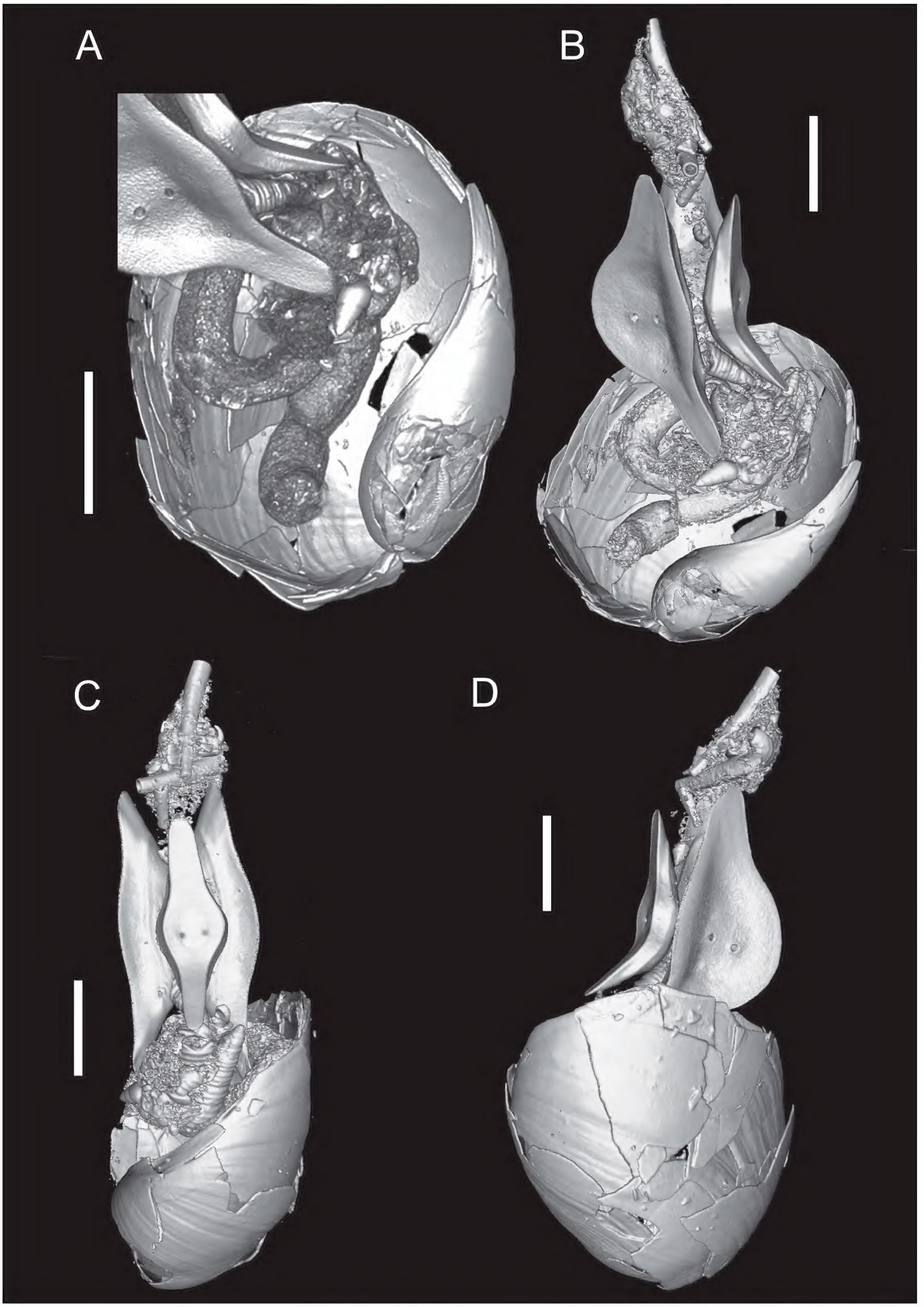

Figure 2. Micro-CT reconstruction images of C.559479 **A** ventral view of the shell **B–D** three perspectives from the reconstruction. Scale bar: 5 mm (**A–D**).

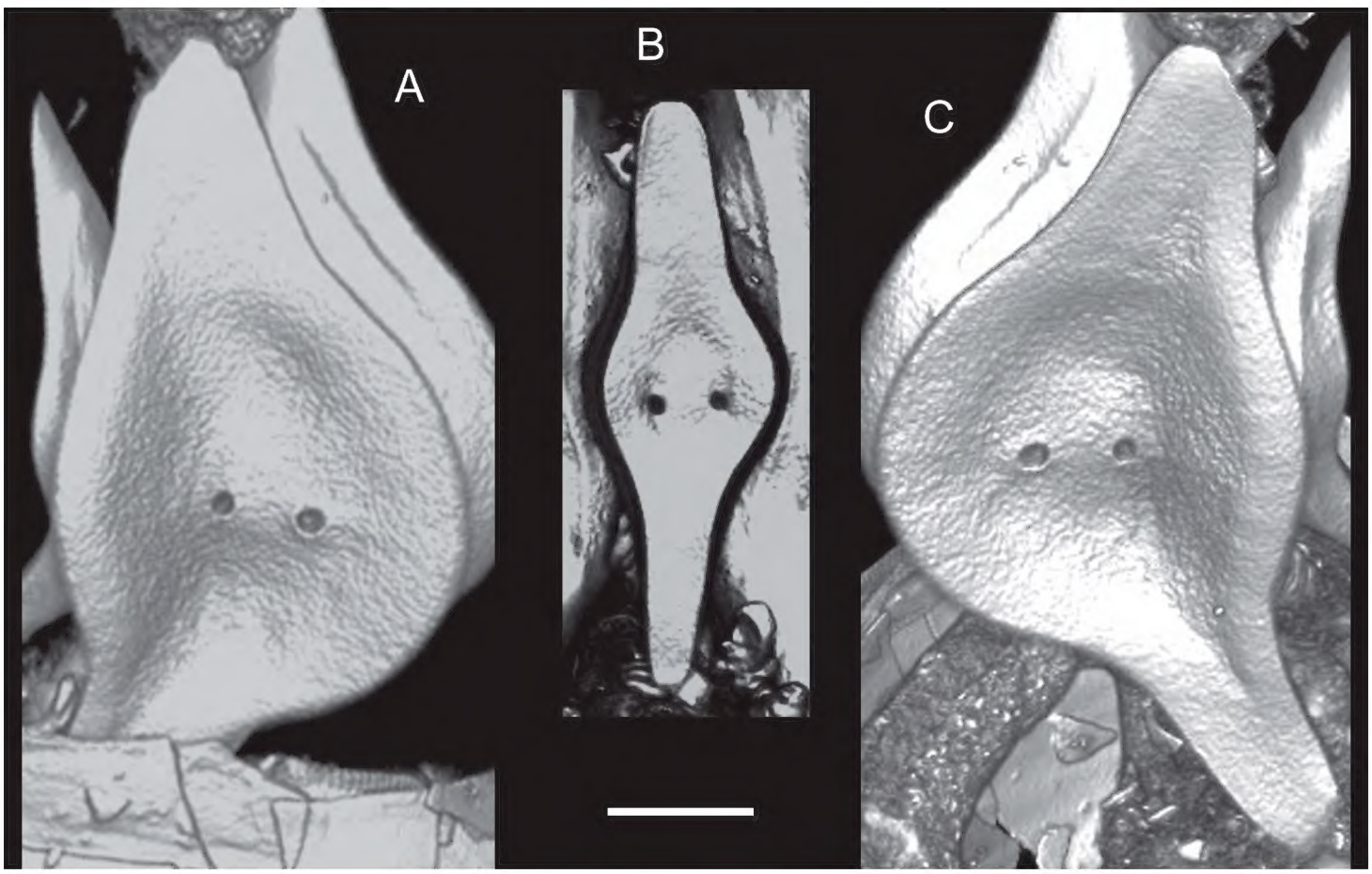

Figure 3. C.559479, outer surface of gizzard plates from the micro-CT reconstruction **A** paired plate, left **B** unpaired plate **C** paired plate, right. Scale bar: 2 mm (**A–C**).

snails (Taylor 1982). The diet of C.559479 included multiple species of micro-gastropods (at least three are observable in Fig. 2B, including the sparsely pustulated micro-gastropod between the gizzard plates) and scaphopods (at the mouth in Fig. 2C and in the Suppl. material 1). We are not aware of previous reports of Philinidae feeding on this class of molluscs.

DNA

The 16S rRNA alignment comprised 444 positions of which 38 were variable but not parsimony-informative and 169 which were parsimony-informative. The optimal tree found in the ML analysis of the alignment had a *ln* likelihood of –3619.22 and there were 650 bootstrap replicates conducted under the MRE criterion. After Gblocks filtering, there were 370 positions in the alignment, of which 30 were variable but not parsimony-informative and 133 were parsimony-informative. ML analysis of these data had a *ln* likelihood of –2831.37 and there were 800 bootstrap replicates conducted under the MRE criterion. The optimal tree found in the ML analysis of the reduced 16S rRNA dataset (Fig. 4) had a *ln* likelihood of –1787.45 and 500 bootstrap replicates were conducted under the MRE criterion. In the optimal trees based on the Gblocks-filtered data and the reduced dataset, the sequence of C.559479 was resolved but without bootstrap support as the sister group of a clade of three sequences apparently, as discussed below, from *Philine quadripartita* Ascanius, 1772. The sequence of C.559479 and the three *P. quadripartita* sequences formed two separate basal lineages

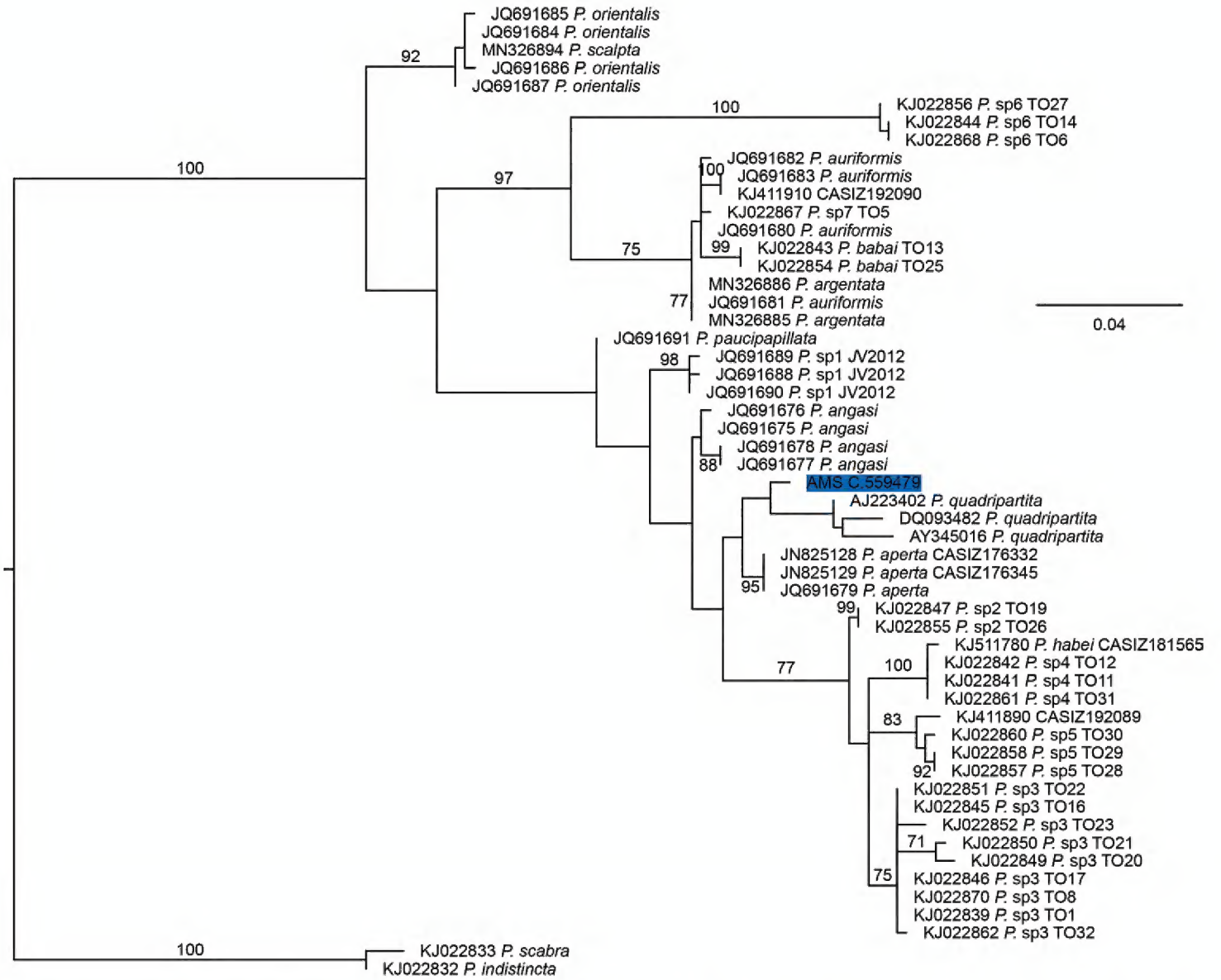

Figure 4. Phylogenetic relationships of C.559479 based on maximum likelihood analysis of the reduced 16S rRNA dataset. More distant outgroups have been removed and the topology rooted on *Philine scabra* + *P. indistincta*. Numbers near nodes refer to bootstrap percentages above 70%. The scale bar indicates 0.05 changes per site. Sequences are identified by accession number and species name or informal designation recorded in GenBank except those labelled *P. quadripartita* for which the species names have been changed for reasons outlined in the text. Accessions with an sp. number designation followed by a space and "TO" with a one or two digit designation refer to sequences from Oskars et al. (2015). Note that this article refers to the undescribed species in alphabetical rather than numerical order so that sp. 4 in GenBank is identified as sp. D in Oskars et al. (2015).

in a large clade that received bootstrap support of 56% in the analysis of the complete 16S rRNA alignment. The K2P distances from C.559479 to the three *P. quadripartita* specimens averaged 0.027 (minimum 0.023).

In all analyses of the 16S rRNA data, MN326885 collected from *P. argentata* by Chaban et al. (2019) and the two *P. auriformis* Suter, 1909 sequences form a clade close to other specimens from the former species. Price et al. (2011) proposed that *P. argentata* is a synonym of *P. orientalis* Adams, 1854. The type specimen of the former has shell dimensions of 6 × 5 mm (Gould 1859) which is much smaller than those of the latter species. *Philine argentata* was maintained as a distinct species by Chaban (2014) owing to differences in gizzard plate morphology, and subsequently by Cha-

ban et al. (2019). The Chaban et al. (2019) sequence of *P. scalpta* A. Adams, 1862 (MN326894) was unexpectedly included with robust bootstrap support in a clade of sequences supposed to be from *P. orientalis* that were collected by Krug et al. (2012).

The 28S rRNA D1 alignment (Suppl. material 2: Fig. S1) comprised 337 bases, 300 of which were constant, 15 parsimony-uninformative and 22 parsimony-informative. The optimal ML topology had a *ln* likelihood of –832.825 based on 1000 bootstrap replicates. The sequence of C.559479 was most similar to the only available sequence of *P. aperta* (Linnaeus, 1767) (K2P distance of 0.003, with the next most similar sequence having a distance of 0.006). The C.559479 and *P. aperta* specimens were not resolved as sister groups in the ML analysis but did have this relationship in minimum evolution topologies (not shown). 28S rDNA D1 sequences were not available from *P. quadripartita*.

The histone H3 alignment (Suppl. material 3: Fig. S2) comprised 365 bases, of which 258 were constant, 23 parsimony-uninformative and 84 parsimony-informative. The optimal ML topology had a *ln* likelihood of –1819.273 based on 500 bootstrap replicates conducted under the MRE criterion. The sequence of C.559479 was included in a large clade (bootstrap support 81%) with sequences from *P. scalpta*, *P. japonica*, and five undescribed species (Oskars et al. 2015) from the Philippines, southwest Pacific, and Taiwan (one specimen: accession KJ022956). The latter specimen was collected from deep water (326–331 m) off northeastern Taiwan (24°48'22.8024"N, 122°07'58.206"E). The most similar sequence to C.559479 was from Panglao in the Philippines (KJ022971, K2P distance 0.012), with the next most similar (K2P distance 0.015) being from *P. aperta* (DQ093508) and the Taiwanese specimen.

Discussion

Morphologically, C.559479 has more apparent affinity with *P. otukai* Habe, 1946, than other Taiwanese species. If the taxa were conspecific, this would represent a very large increase in the depth range of *P. otukai* which is reported to extend only to 100 metres (Lin 2004). The definite identification of C.559479 as *P. otukai* would, however, be premature because of the lack of relevant morphological information for that species. The shell of *P. otukai* is generally larger than that of C.559479, with the type specimen (Otuka 1936: figs 22, 23, as "*P. aperta*") of the former having a length of 24 mm and width of 17 mm (Otuka 1936). Although Lee (1998) gives a minimum of 15 mm (in a range of 15–28 mm) most reports from other regions (e.g., Lin 2004) suggest that the shell is much longer (> 25 mm). The gizzard plates of C.559479 appear similar in shape to those of *P. otukai* illustrated by Lee (1998) depicting the inner surface. However, no pores are visible in the illustrations of the outer surfaces of the plates in Lee (2018).

Specimen C.559479 is readily distinguished from *P. argentata*, which has two slit-like recesses on the outer surfaces of its gizzard plates (Chaban and Chernyshev 2014), that are absent here (Fig. 3). *Philine argentata* and *P. orientalis* were synonymised by (Price et al. 2011). However, they were treated as distinct species by Chaban (2014) so the possibility that C.559479 belongs to *P. orientalis* may be considered. The specimen C.559479 is dis-

tinct externally from *P. orientalis* sensu (Price et al. 2011) in its smaller size and creamy yellow headshield colour (vs. white in the latter species). C.559479 has a large pair of circular pores on all gizzard plates, whereas the pores are small in *P. orientalis* Price et al. (2011).

Specimen C.559479 differs from *P. vitrea* in the small size of the latter (shell dimensions of the type specimen 10 × 8 × 3 mm: Gould 1859). It also differs in the apparently much lower projection of the upper end of the outer lip of *P. vitrea*, allowing for the damaged condition of this shell section in the specimen studied here.

Sufficient information is available to distinguish C.559479 morphologically from only a few other regional species of *Philine*. These include *P. scalpta* in which the gizzard plates are quasi-trapezoidal with elongated pores (Chaban and Chernyshev 2014). The shell of the shallow water *P. kinglipini* Tchang, 1934 differs by the absence of spiral sculpture (Lin 1997: 198). *Philine kinglipini* attains a larger size of ~ 40–42 mm length and 18–19 mm width with shell dimensions of 19–20 mm × 14–14.5 mm (Lin 1997). The gizzard plates of *P. kinglipini* are all dissimilar to each other in size (Tchang 1934), with the second largest being quasi-trapezoidal. Both arms on all three plates of *P. kinglipini* are broad and nearly equal in length.

Philine paucipapillata Price, Gosliner & Valdés, 2011 was described as a new species from the South China Sea. Specimen C.559479 is readily distinguished morphologically from this species in coloration and size (*P. paucipapillata* is uniformly white and ~ 4–5 cm in length). The pores in the gizzard plates of *P. paucipapillata* are minute (Price et al. 2011) in contrast to those of C.559479.

Philine kurodai Habe, 1946, described from Japan, has been reported from Taiwan although the Taiwanese records are now regarded as being based on specimens of *P. otukai* (Lee 1998; Wu 2004). However, although both *P. otukai* and *P. kurodai* were described in Habe (1946) no characters distinguishing them from each other were listed and we have been unable to find any subsequent discussion discriminating these species. Neither *P. kurodai* nor *P. otukai* was mentioned by Price et al. (2011) but both are accepted by Chaban (2016).

Three species from northeastern Asia, *P. acutangula* Adams, 1862, *P. crenata* Adams, 1862, and *P. coreanica* Adams, 1855, have received little recent attention, particularly after Herdman (1906) made the latter two synonyms of "*Philine aperta*" (Table 1). The species all appear to belong to the group of species described by Adams (1855, 1862) categorised by Kurodai and Habe (1954) as "unobvious" and are presently best regarded as species inquirenda. To the best of our knowledge, the locations of their type specimens are unknown.

Analyses including the DNA sequences of C.559479 collected here highlight the taxonomic complexity of *Philine* in northeastern Asia. The available 16S rRNA data from the region represent at least five species. One of these species is represented by the C.559479 sequence from Taiwan. The other species are *P. paucipapillata*, *P. argentata* sensu Chaban et al. (2019), a species uncertainly referred to "*P. orientalis*", *P. japonica* or *P. scalpta* – see below), and an additional species, denoted "species D" by Oskars et al. (2015), which is represented by histone H3 DNA sequences. The latter may have affinity with *Philine habei* Valdés, 2008. The Taiwanese specimen of "species D" lacks 16S rDNA sequences but the other specimens referred to it by Oskars et al. (2015) are found in analyses of this

gene (Fig. 4) in a strongly supported clade with the *P. habei* sequence (KJ511780). This clade clearly excludes C.559479. The gizzard plates of *P. habei* have large elongate pores (Valdés 2008; Price et al. 2011) that appear very different from those of C.559479.

The three sequences of 16S rRNA that were most similar to C.559479 are recorded in GenBank as belonging to *Philine aperta*. However, the true identity of the specimens from which these sequences were derived appears to be *P. quadripartita*, a species from the northern Atlantic Ocean and Mediterranean Sea. The sequences form a distinct lineage separate from the two South African specimens from near the type locality of true *P. aperta*, and were determined from specimens taken within the range of *P. quadripartita*: AY345016 derives from a specimen from south-eastern Spain (Grande et al. 2004); and both AJ223402 (Thollesen 1999) and DQ093482 (Aktipis et al. 2008) are from Sweden.

The "*P. orientalis*" 16S rRNA sequence accession JQ691684) from Japan (Krug et al. (2012) is from a specimen identified as *P. japonica* in the catalogue of the Natural History Museum, London (BMNH:1996409). This is included in a clade with three Californian (non-native range) haplotypes of "*P. orientalis*" sequenced by Krug et al. (2012) and another from a specimen identified by Chaban et al. (2019) as *P. scalpta*, a species that was redescribed by Chaban and Chernyshev (2014) who removed it from the synonymy of *P. vitrea* in which it had been placed by Higo et al. (1999). If *P. japonica* and *P. scalpta* are, as maintained by Chaban et al. (2019), distinct from *P. orientalis*, then sequences truly from the latter species are probably yet to be determined. It is possible that the sequences from the taxon from near Darwin in northern Australia labelled sp. 1 JV 2012 in GenBank (Krug et al. 2012) may belong to *P. orientalis* or a closely related species. The named species to which these sequences are most similar are the Australasian *Philine angasi* (Crosse, 1865, in Crosse and Fischer 1865) and *P. paucipapillata*. Some radular and penial morphology characters distinguish *P. paucipapillata* and *P. orientalis* but they are closely related in the morphologically based phylogeny of Price et al. (2011). In this phylogeny, *P. paucipapillata* is shown as the sister group of the pair composed of *P. orientalis* and *P. angasi*.

Conclusions

The difficulty of identifying the unusual deep water *Philine* specimen studied here emphasises that destructive sampling should be minimised where the taxonomy is unintegrated. We tentatively suggest from examination of the external morphology and micro-CT scanning that it may have affinities with the shallow water *P. otukai* but any certainty is precluded by the lack of a description of that species, especially one detailing the range of variation it might encompass in characters such as the size of the gizzard plate pores. There is no diagnosis of *P. otukai* that would guide specimen dissection for relevant anatomical characters. Considerable further research efforts are required to provide a framework in which such characters could be sought. These include DNA studies of a wider range of regional species of *Philine*, particularly sequences definitely from *P. orientalis*, *P. kurodai*, *P. otukai*, and *P. vitrea*. Detailed morphological investigations are

needed for *P. japonica*, *P. otukai*, *P. kurodai*, and *P. vitrea*. The status of species such as *P. coreanica* may remain unknown but it may possibly be resolved if shell fragments from type material ever become available for macromolecular analysis, especially proteomics.

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Supplementary material 1

Reconstructed micro-CT scan of C.559479

Authors: Donald J. Colgan, Shane T. Ahyong, Karine Mardon, Ian M. Brereton

Data type: Mpg file

Explanation note: Reconstructed micro-CT scan of C.559479.

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Link: https://doi.org/10.3897/zookeys.1060.28809.suppl1

Supplementary material 2

Figure S1. Image from the reconstructed micro-CT scan of C.559479

Authors: Donald J. Colgan, Shane T. Ahyong, Karine Mardon, Ian M. Brereton

Data type: Pdf file

Explanation note: Image from the reconstructed micro-CT scan of C.559479. Dorsal view of the shell of C.559479, with boxed area illustrating irregularly cancellate sculpture.

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Link: https://doi.org/10.3897/zookeys.1060.28809.suppl2

Supplementary material 3

Figure S2. Phylogenetic relationships of C.559479 based on maximum likelihood analysis of the D1 28S rRNA dataset

Authors: Donald J. Colgan, Shane T. Ahyong, Karine Mardon, Ian M. Brereton

Data type: Pdf file

Explanation note: Phylogenetic relationships of C.559479 based on maximum likelihood analysis of the D1 28S rRNA dataset. Numbers near nodes refer to bootstrap percentages above 70%. Scale bar indicates 0.01 changes per site. Sequences are identified by accession number and species name or informal designation recorded in GenBank. Accessions with an sp. number designation followed by a space and "TO" with a one or two digit designation refer to sequences from Oskars et al. (2015). Note that that article refers to the undescribed species in alphabetical rather than numerical order so that sp. 4 in GenBank is identified as sp. D in Oskars et al. (2015).

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Link: https://doi.org/10.3897/zookeys.1060.28809.suppl3

Supplementary material 4

Figure S3. Phylogenetic relationships of C.559479 based on Maximum Likelihood analysis of the histone H3 dataset.

Authors: Donald J. Colgan, Shane T. Ahyong, Karine Mardon, Ian M. Brereton

Data type: Pdf file

Explanation note: Phylogenetic relationships of C.559479 based on Maximum Likelihood analysis of the histone H3 dataset. Numbers near nodes refer to bootstrap percentages above 70%. The scale bar indicates 0.05 changes per site. Sequences are identified by accession number and species name or informal designation recorded in GenBank. Accessions with a sp. number designation followed by a space and "TO" with a one or two digit designation refer to sequences from Oskars et al. (2015). Note that this article refers to the undescribed species in alphabetical rather than numerical order so that sp. 4 in GenBank is identified as sp. D in Oskars et al. (2015). The sequence indicated by a red square is the Taiwanese specimen with apparent affinity to P. habei (see text).

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